

# Activity Patterns and Parasitism Rates of Fire Ant-Decapitating Flies (Diptera: Phoridae: *Pseudacteon* spp.) in Their Native Argentina

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Ann. Entomol. Soc. Am. 101(3): 539–550 (2008)

**ABSTRACT** This work describes the annual and daily activity patterns of two parasitoid fly communities of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in their native range in Argentina. *Pseudacteon* (Diptera: Phoridae) flies were censused monthly for 1 yr at two sites in northwestern Corrientes province. Additionally, parasitism rates of *S. invicta* colonies naturally attacked in the field by *Pseudacteon* flies are reported for the first time from its native habitat. In total, 4,528 flies (86.3% females) of eight *Pseudacteon* species were collected attacking ants in the field. *Pseudacteon litoralis* Borgmeier and *Pseudacteon nocens* Borgmeier represented 71–79% of female flies censused in both sites. Most species were active throughout the year, although abundances were variable over time and between sites. The highest occurrence peaks of flies were recorded in spring, whereas the lowest occurrence was in summer. Fly abundance was higher close to dusk, whereas species diversity was highest at midday. Relationships among species were established based on their activity patterns and genetic proximity. The presence and abundance of four species were explained by climatic variables, whereas two species may have inherited similar circadian rhythms from a common ancestor. Overall, the parasitism rate by the nine species recorded was very low (0.24%). The highest percentage of parasitized workers was found in spring (0.5%) and occurred within the most complex habitat (gallery forest). The highest parasitism rate per site and colony was also for this habitat in spring (1.16 and 2.81%, respectively). The highest rates of emergence were recorded for *Pseudacteon nudicornis* Borgmeier.

**KEY WORDS** phenology, species relationships, *Solenopsis invicta*, climate, biological control

Daily and seasonal rhythms are intrinsic to virtually all life forms. In insects, as in many other organisms, the most evident rhythms are related to behaviors such as locomotion, flight, foraging, and oviposition. These behaviors are usually restricted to certain times of the day, because they are often synchronized with periodic events in the environment such as light and temperature cycles (Giebultowicz 2000), or more episodic events related to food availability, predation, and competition (Kronfeld-Schor and Dayan 2003). Thus, the environment directly or indirectly regulates the occurrence and abundances of terrestrial organisms such as insects in nature (Wolda 1988). Temperature and moisture are the most widely recognized climatic variables controlling arthropod presence and abundance.

External cues provide the information that insects need to synchronize their daily and seasonal behaviors (Dunlap 1999). However, daily behavioral rhythms

also persist in constant environmental conditions because the existence of endogenously timing mechanisms, known as circadian clocks (Giebultowicz 2000). These genetic mechanisms have been discovered in organisms ranging from bacteria to humans (Dunlap 1999, Giebultowicz 2000, Wagner-Smith and Kay 2000). Despite having this internal chronometer, many insects' circadian rhythms must still be synchronized with the environment (Dunlap 1999, Giebultowicz 2000).

Although some insects, such as the fruit fly, *Drosophila melanogaster* Meigen, have been a successful model to study circadian rhythm mechanisms (Giebultowicz 2000), little is known about phenological patterns in other insect groups. The dipteran family Phoridae is a clear example (Disney 1994). The Phoridae is one of the most biologically diverse families of insects, and large numbers of phorid species are parasitoids or specific predators (Disney 1994). Phorids include the only group of dipteran parasitoids that has radiated extensively on social insect hosts, ants in particular (Disney 1994). The few studies of circadian and circannual rhythms in this dipteran family were conducted almost exclusively on the *Pseudacteon* fire ant decapitating flies (Disney 1994) because of the flies potential as biological control agents of the imported fire ants *Solenopsis invicta*

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Buren and *Solenopsis richteri* Forel in the United States (Porter and Gilbert 2004). Phorid flies indirectly affect fire ants by reducing their ability to retrieve resources by exploitation competition with native ants (Feener and Brown 1992, Orr et al. 1995, Folgarait and Gilbert 1999, Feener 2000, Calcaterra et al. 2008). Because of this detrimental effect, four species have been released against fire ants in the southern United States (Porter and Gilbert 2004, Patrock et al. 2008).

Approximately 30 species of fire ant decapitating flies occur in North and South America. Of these species, at least 22 attack South American fire ants in the *Solenopsis saevissima* species-group (Porter and Pesquero 2001, Calcaterra et al. 2005, Folgarait et al. 2005, Calcaterra 2007). Most of the fly species are distributed across a wide range of habitats and climates (Folgarait et al. 2005; Calcaterra et al. 2005, 2007). Assemblages of up to nine fly species attacking *S. invicta* have been found at a single site in Corrientes, eastern Argentina (Calcaterra et al. 2005) and up to 11–12 species in a wider area in Santiago del Estero, north central Argentina (Azzimonti et al. 2004, 2005).

Temporal partitioning (or differential activity patterns) among competitor species (Wiens et al. 1986) is one of the main factors promoting the coexistence of these sympatric fly species, because different fly species are known to be active during different seasons and at different times of the day in Brazil (Fowler et al. 1995, Pesquero et al. 1996), the United States (Morrison et al. 1999, Wuellner and Saunders 2003, Morrison and Porter 2005), and Argentina (Folgarait et al. 2003, Azzimonti et al. 2004, 2005).

Although the phenology of fire ant decapitating flies is known from the above-mentioned studies, little information is known about activity patterns of *Pseudacteon* fly assemblages attacking populations of the red imported fire ant. The use of fly species or biotypes adapted to attack *S. invicta* with complementary activity patterns (and/or wide phenotypic plasticity) would have a higher impact on the red imported fire ant populations in the United States (Porter and Gilbert 2004).

The main objective of this work was to examine the annual and daily activity patterns of two parasitoid fly communities of *S. invicta* in northeastern Argentina. Activity patterns observed in the field were placed in the context of climatic variables and genetic relationships among fly species. Additionally, parasitism rates of fire ant colonies (mostly *S. invicta*) naturally attacked in the field by *Pseudacteon* flies are reported for the first time from South America.

## Materials and Methods

**Temporal Activity Pattern.** Activity of flies was monitored monthly from January to December 2005 in two sites with moderate anthropogenic modification: 1) National Aquaculture Development Center (NADC; 27° 23' S, 58° 41' W) and 2) Corrientes Biological Station (CBS; 27° 33' S, 58° 41' W). The two sites are 20 km apart, in a mesic environment in the Chaqueña phytogeographical province (Cabrera and

Willink 1980). The monitored area in each site was ≈1 ha. The relative abundance of flies was recorded in each area by attracting phorid flies to five trays (7 by 24 by 37 cm) 3–15 m apart, each containing 3–5 g of *S. invicta* workers and 1–3 g of brood. The walls of the trays were coated with Fluon (polytetrafluoroethylene; ICI Floropolymers, Exton, PA) to prevent workers from escaping. A small inverted nest box, made of wood and plaster, was included in each tray as a refuge for workers and brood. The wooden boxes were moved every 5 min to keep the workers in motion. The five trays were exposed for 30 min (sampling effort) every 3 h from sunrise until sunset (sunrise, 1000 hours, 1300 hours, 1600 hours, sunset) on three consecutive days. All the flies attracted to the trays were aspirated, identified, counted, and then released at their original collecting site or preserved in 96% alcohol for later genetic analyses. Depending on the number of flies captured (censused), a 20× hand-held lenses or a portable dissecting microscope was used to identify female flies with the key of Porter and Pesquero (2001) key. Species-level determination in the field was not possible for males because of the absence of keys, but most, if not all, were either *Pseudacteon tricuspidis* Borgmeier or *Pseudacteon obtusus* Borgmeier, because males of other species are not normally attracted to ants (Calcaterra et al. 2005). Females of all the species usually go to the mounds (or trays with many workers), for oviposition. The only exception is *Pseudacteon solenopsidis* (Schmitz), whose females only attack isolated workers in foraging trails (Wuellner et al. 2002, Calcaterra et al. 2005). The average number of flies captured in each period was recorded.

**Climatic Variables.** Air temperature and relative humidity during the sample period were measured in the shade ≈1 m above the ground with a portable Kestrel weather station. Long-term climatic data (20-yr records, Servicio Meteorológico Nacional 1984–2004) were obtained from Corrientes meteorological station (27° 28' S, 58° 49' W). According to the 20-yr historical records, the area has a mean annual temperature of 21.4°C and annual rainfall of 1,652 mm. The climate is seasonal with drier winters. Temperature and humidity during the period of study (2005) also were recorded using a HOBO data logger. Meteorological and climatic variables were used to try to explain fly occurrence through time as in Folgarait et al. (2003). Historical variables (mean monthly temperature, maximum and minimum mean monthly temperature, mean monthly rainfall) and those taken during the study period (mean monthly temperature, mean monthly relative humidity, monthly rainfall; and mean temperature, mean humidity, and accumulated rainfall in the previous 2 mo before the sampling time) were used.

**Molecular Analysis.** *Pseudacteon* flies for analysis were collected from the surface of *S. invicta* colonies at NADC and preserved in 96% alcohol. *Pseudacteon curvatus* Borgmeier, *Pseudacteon nocens* Borgmeier, *Pseudacteon cultellatus* Borgmeier, *P. tricuspidis* Borgmeier, *Pseudacteon nudicornis* Borgmeier, *Pseudacteon*

**Table 1.** Parasitism rates of *Pseudacteon* flies on *Solenopsis* workers in Machuca campground (MC), Concepción del Uruguay (CU), Corrientes Biological Station (CBS), and Herradura (HF)

Sites <sup>a</sup>	Nov. 2004			Feb. 2005			April 2005			Total rates
	No. fly pupae	No. host workers	% parasitism	No. fly pupae	No. host workers	% parasitism	No. fly pupae	No. host workers	% parasitism	
MC										
Mean	38.5	3,519	1.16	5.50	3,926	0.12	12.0	4,426	0.26	0.5
SD	34.7	494	1.08	4.04	1,282	0.08	8.6	800	0.16	
Range			0.34–2.81			0–0.23			0.13–0.53	
CU										
Mean	13.5	2,948	0.42	1.25	3,494	0.05	0	3,888	0	0.15
SD	22.6	459	0.66	1.58	987	0.08		878		
Range			0–1.87			0–0.23				
CBS										
Mean	10.5	3,534	0.30	3.88	3,517	0.12	3.0	4,618	0.06	0.15
SD	9.9	318	0.27	2.17	560	0.08	2.9	533	0.07	
Range			0.05–0.84			0.03–0.24			0–0.21	
HF										
Mean	4.5	3,114	0.16	0	2,869	0	1.8	3,836	0.05	0.10
SD	4.6	371	0.17		467		3.0	591	0.09	
Range			0–0.04						0–0.22	
Total	536	104,919	0.51	87	93,231	0.09	116	113,495	0.10	0.24

<sup>a</sup> Eight fire ant colonies were sampled from each site in each season, with the exceptions of Herradura in February ( $n = 2$ ) and April 2005 ( $n = 5$ ), and Machuca Campground in May ( $n = 7$ ).

*litoralis* Borgmeier, and both forms of *P. obtusus* were collected in April 2004, whereas *P. solenopsidis* was collected in December 2004.

To reconstruct relationships within and among *Pseudacteon* species, genomic DNA was extracted following the protocol of Sunnucks and Hales (1996), as described in Calcaterra et al. (2007). We then performed polymerase chain reaction (PCR) by using the primers CI-J-2183 (Jerry) and TL2-N-3014 (Pat) for the mitochondrial cytochrome oxidase I gene (COI) (Simon et al. 1994). The reaction was performed as described in Calcaterra et al. (2007), we checked reaction products by visualization on agarose gels, and purified PCR products for DNA sequencing by digesting with exonuclease I and shrimp alkaline phosphatase. We sequenced the PCR products using the Applied Biosystems (ABI, Foster City, CA) Big Dye 3.1 cycle sequencing kit and ran the products on an ABI 3100 Genetic Analyzer, as described in Calcaterra et al. (2007). Resulting sequences were aligned using Clustal W (Thompson et al. 1997) and verified by eye. Additional sequences of *P. obtusus* from Calcaterra et al. (2007) were trimmed to match the length of the COI sequences generated from the above-mentioned samples, aligned, and included in the analysis. Phylogenetic relationships were reconstructed using maximum parsimony and maximum likelihood, as implemented in PAUP (Swofford 1998), and estimated support by performing 1,000 bootstrap replicates. COI sequence of a syrphid fly, *Chalcosyrphus nemorum* (F.) (Diptera: Syrphidae) (GenBank accession no. AY261701), was used as the outgroup (Stahls et al. 2003). The analysis also was performed without outgroup and midpoint rooting the tree (data not shown); the overall topology did not differ from the results with the outgroup.

**Natural Parasitism Rates.** Rates of natural parasitism were estimated in November 2004 (spring), February

2005 (summer), and April 2005 (early fall), in four selected sites with high fly abundance (Calcaterra et al. 2005). The four sites were 1) Machuca campground (MC; 27° 23' S, 58° 41' W) and 2) Corrientes Biological Station (CBS), Corrientes; 3) Herradura (26° 31' S, 58° 17' W), Formosa, where *S. invicta* is the most common species; and 4) Concepción del Uruguay (32° 28' S, 58° 14' W), Entre Ríos, where *S. richteri* is the most common fire ant species. In each site, eight mounds were excavated (fewer numbers of colonies were sampled in a few occasions) to obtain a representative worker sample from the colony (Table 1). Although mounds were chosen at random, it is possible that some colonies were sampled more than once because the fire ant colonies move their mounds very often (Briano et al. 1995). Colonies were transported to the laboratory and workers separated by flotation. A subsample of several thousand workers (2–3 g) of each ant colony was taken randomly, placed in plastic rearing trays (24 by 13 by 5 cm), and maintained at 25–27°C with a photoperiod of 12:12 (L:D) h. A plaster block saturated regularly with water was placed in each tray to maintain humidity levels near 100%. The ants were fed sugar-water solution ad libitum. The trays were inspected every 1–3 d for 50 d for the appearance of pupae (and larvae) in the heads of dead ants. Pupae (and larvae) were transferred to plastic containers with moistened plaster in the bottom until flies emerged. The numbers of pupae (plus larvae) and viable offspring were recorded for each tray. Flies were identified to species under a dissecting microscope. Parasitism rate was calculated as the total number of pupae found per tray divided by the total estimated number of workers per tray.

**Statistical Analysis.** Overall fly abundance in each site was compared with a one-way analysis of variance (ANOVA). The mean number of flies was log<sub>10</sub> transformed to normalize the data. Pearson's product-mo-

ment correlation was used for comparing fly activity patterns. *Pseudacteon* species were grouped using a cluster analysis with fly species activity data (correlation index). This analysis was performed using the unweighted pair-group average algorithm (unweighted pair-group method with arithmetic average). Single or multiple (forward stepwise) linear regressions were used to evaluate if the fly activity patterns observed at each site are explained by meteorological and climatic variables. Principal component analysis (PCA) was used to identify variables that better explains the climatic variation. PCA was applied on the correlation matrix of the climatic variables gathered through the time.

## Results and Discussion

**Overall Annual Activity Pattern.** In total, 4,528 flies of eight *Pseudacteon* species were recorded in CBS and NADC. At CBS, 91.7% (1,728/1,885) were females, whereas 82.6% (2,182/2,643) were females at NADC. Overall fly abundances were variable between sites and over time. Flies were 40% more abundant at NADC than at CBS (2,643 versus 1,885 flies, respectively), but the difference in mean monthly fly abundance between sites was not significant ( $F = 1.33$ ;  $df = 1, 22$ ;  $P = 0.26$ ). The mean number of flies per sampling effort (five trays containing ants exposed during 30 min) was  $15.6 \pm 18.7$  flies (range 0–121) at NADC and  $10.4 \pm 13.3$  flies (range 0–86) at CBS. This value is higher than the one reported by Folgarait et al. (2003) for a fly assemblage compounds by six *Pseudacteon* species in Buenos Aires (<10 flies per h).

Monthly changes in overall fly abundance were uncorrelated between sites ( $r = 0.25$ ,  $P > 0.42$ ). Although the pattern was similar between January and May at both sites, it differed during the rest of the year. The highest abundance of flies was recorded in spring, whereas they were least abundant during the summer. The highest number of flies was censused in December at NADC (576 flies) and November at CBS (364 flies), whereas the lowest one was in February at NADC (50 flies) and January at CBS (39 flies). The second highest abundance peak at CBS was in July; but fewer flies were censused between July and October.

At NADC, the fly occurrence increased month to month up to December.

The high fly occurrences recorded in spring in Corrientes agree with those reported by Azzimonti et al. (2004, 2005) for Santiago del Estero. They found the highest abundances in spring and fall and the richest of species (11) in March (summer). Fowler et al. (1995) also found the highest occurrence of flies in Brazil in spring, but attacking another fire ant species, *Solenopsis saevissima* (Smith). As suggested by Fowler et al. (1995), the high fly occurrences during spring might be due to the highest host colony activity at that time by causing the mating flights. However, this spring peak also could be explained by the start of the rain season. Many more larvae would reach to the pupa stage when the humidity increases by cause of the rains. Although the seasonality of fly populations

might have not been accurately represented due to limited sampling period (1 yr), differences in abundance patterns among years and sites also were reported by Morrison et al. (1999) for five populations of four native *Pseudacteon* species attacking *Solenopsis geminata* (F.) in the United States.

**Climatic Variables.** The mean temperature during the year of study was similar to the historical records: 20.8°C, ranging from 37.9°C (January) to -0.6°C (July). However, annual rainfall was lower than average: 1,198.1 mm, ranging from 347 mm (April) to 0.9 mm (September), but similar to the mean rainfall (1,200 mm) reported for eastern Chaco by Cabrera and Willink (1980). This rainfall decrease could have had an effect on the structure of the fly community. The mean relative humidity during 2005 was 76.3%, ranging from 100% (night and sunrise) to 23.2% (mid-day).

The two first components of the PCA of climatic variables across the 12 mo explained 91.02% of the total variance. The variable with the greatest weight in the first component (74.35%) was historical rainfall (5.6), and in the opposite direction, historical minimum mean monthly temperature (-2.7), mean monthly temperature during 2005 and mean temperature 2 mo before records (both with -2.4). The variables with more weight in the second component (16.67%) were accumulated rainfall (-2.7) and mean relative humidity (2.1) both 2 mo before records. Five of these six climatic variables were chosen to explain the fly activity patterns. Mean monthly temperature during 2005 was excluded because it strongly correlated with mean temperature two months before records ( $r = 0.95$ ,  $P = 0.0000$ ).

**Annual Activity Patterns of Individual Species.** Despite a large variation, annual activity patterns were observed for seven *Pseudacteon* species. Because *P. curvatus* was collected only in March, it was considered an outlier and excluded from the analysis. The absence of *P. curvatus* in the two assemblages studied was surprising, because it had been previously reported from several sites in this region of Argentina (Calcaterra et al. 2005, Folgarait et al. 2005). The reasons for its absence remain unknown; however, it could be due the host ants in the two study sites are not suitable for *P. curvatus* or the competitive exclusion of *P. curvatus* by some of the smaller flies, such as *P. cultellatus*.

Most fly species were active throughout the year, although most of them differed in their abundance patterns (Fig. 1). Most species showed more than one peak of abundance. Several species showed similar annual abundance fluctuations at NADC or CBS. Similarity among species in each site is represented in Fig. 2. *P. litoralis* and *P. nocens* had the most similar pattern at NADC ( $r = 0.95$ ,  $P = 0.0000$ ) and CBS ( $r = 0.90$ ,  $P < 0.0001$ ), although their relationships with the other species were different at each site. These two species represented 71–79% of the female flies censused at NADC and CBS. *P. nocens* also has been reported as the most abundant fly species throughout the year in six sites in Santiago del Estero (Azzimonti et al. 2004).

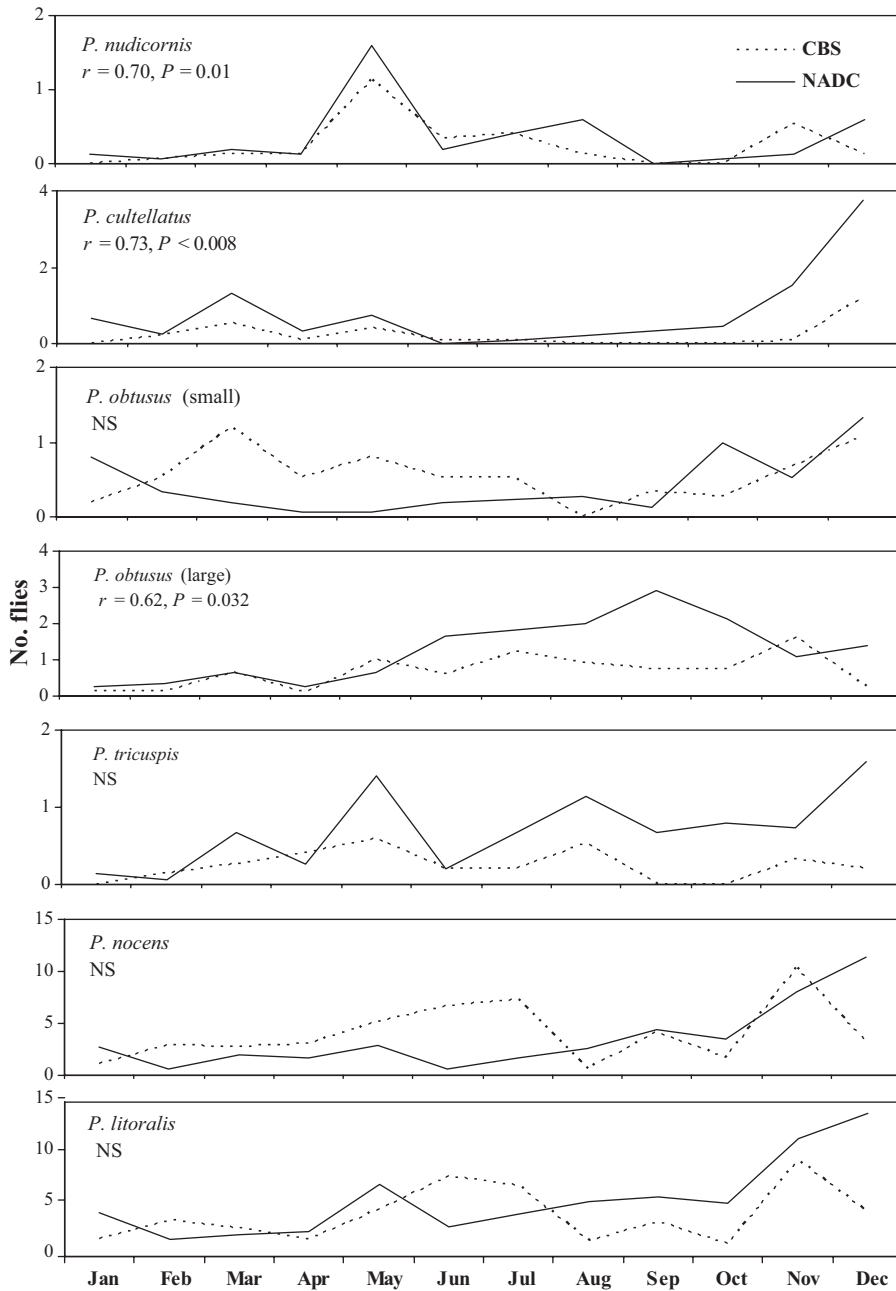


Fig. 1. Annual activity pattern of the seven *Pseudacteon* species studied. Note differences in scale of y-axes (mean number of flies per sampling effort). Statistical legends refer to the correlations of species activity patterns between sites.

The large *P. obtusus* was the third most abundant species in both sites. These three large fly species were the only species recorded each month in both sites. The second pair of species more similar in CBS was *P. cultellatus* and the small *P. obtusus* ( $r = 0.78$ ,  $P < 0.003$ ). In NADC, the *P. cultellatus* pattern was very similar to the ones of *P. nocens* ( $r = 0.89$ ,  $P < 0.0002$ ) and *P. litoralis* ( $r = 0.79$ ,  $P < 0.002$ ).

Only three species (*P. cultellatus*, *P. nudicornis*, and the large *P. obtusus*) had similar patterns in both sites

(see correlations in Fig. 1). This suggests that their abundance may depend less on the type of habitat than the other four species. The other fly species also were present each month in at least one of the sites, with the exception of *P. nudicornis*, which was absent in September. This species was the least abundant ( $\approx 2.6\%$ ) in both places. *P. cultellatus* and *P. tricuspis* also were scarce at CBS (2.6 and 2.7%, respectively), whereas the small form of *P. obtusus* was scarce at NADC with 3.6%. *P. tricuspis* showed a similar annual



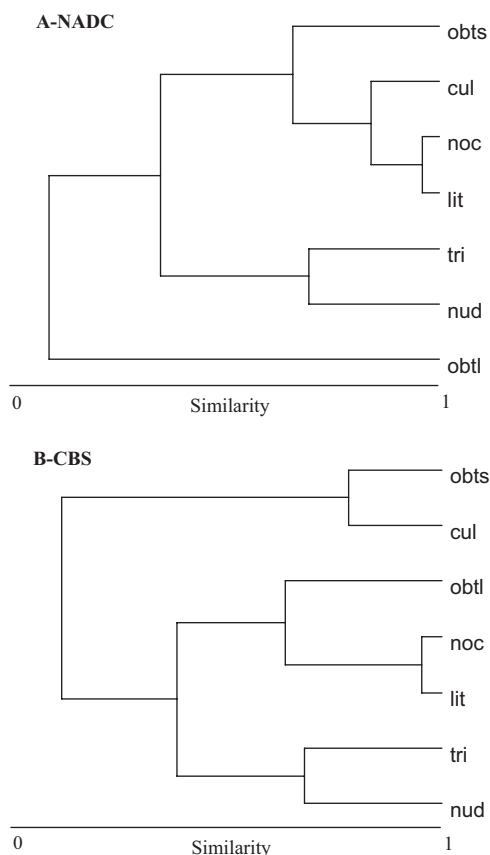


Fig. 2. (A and B) Relationship among the seven studied species in National Aquiculture Development Center (A-NADC) and Corrientes Biological Station (B-CBS) based on correlations of their monthly activity patterns. Fly abbreviations are same as in Table 2.

pattern of abundances for one site (Hogtown Creek) in the United States (Morrison and Porter 2005). Monthly abundance of males only correlated with abundance of *P. tricuspidis* and large *P. obtusus* at NADC ( $r = 0.73$ ,  $P < 0.008$  and  $r = 0.84$ ,  $P < 0.0006$ , respectively) and CBS ( $r = 0.77$ ,  $P < 0.005$  and  $r = 0.69$ ,  $P < 0.012$ , respectively). These correlations are supported by the data that, in the field, only males of these two species were attracted to the nest for mating (Calcaterra et al. 2005).

We found that most of the seven species were active throughout the year in Corrientes, whereas previous studies in Buenos Aires showed that only one of six species of *S. richteri*-decapitating flies was active throughout the year (Folgarait et al. 2003). Although this difference may be due to the limited sampling period (one year) of our study, this discrepancy also could be explained by differences in latitude. The winters are warmer in Corrientes than in Buenos Aires, which likely translates into greater phorid activity throughout the year. The mean temperature of the coldest month (July) in Buenos Aires ( $35^{\circ}$  S) is  $10.3^{\circ}\text{C}$ , whereas in Corrientes ( $27^{\circ}$  S) it is  $15.4^{\circ}\text{C}$  (De

Fina 1992). Flies in Corrientes started flight activity at  $16^{\circ}\text{C}$ .

In CBS, abundance of *P. nudicornis* was best explained by mean temperature and mean relative humidity in the 2 mo before sampling time ( $R^2 = 0.63$ ,  $P = 0.011$ ), whereas abundance of the small *P. obtusus* by historical minimum mean monthly temperature and mean relative humidity in the 2 mo before sampling time ( $R^2 = 0.72$ ,  $P < 0.043$ ). Abundance of the large *P. obtusus* was slightly explained (39%) by mean temperature in the 2 mo before sampling time ( $R^2 = 0.39$ ,  $P = 0.03$ ). In NADC, the abundance of large *P. obtusus* was explained by mean temperature and accumulated rainfall during the 2 mo previous to the sampling time, and historical minimum mean monthly temperature ( $R^2 = 0.83$ ,  $P = 0.002$ ), whereas *P. cultellatus* was partially explained by historical minimum mean temperature ( $R^2 = 0.42$ ,  $P = 0.024$ ). Our findings agree with Folgarait et al. (2003), who found that similar climatic variables explained most of the fluctuations in overall fly abundance in a temperate area in Buenos Aires.

It is important to note that most annual fluctuations in fly abundance were partially explained by variables recorded 2 mo before the sampling time. This is likely the maximum required time by most of the fly species to develop from egg to adult (Porter 1998, Folgarait et al. 2002). The longest developmental time recorded so far for *Pseudacteon* species parasitizing *S. invicta* is 62 d at  $22^{\circ}\text{C}$  (Folgarait et al. 2002). Adequate climatic conditions for each species during this time period would increase the adult emergence. In this study, we found that *P. nudicornis* and both *P. obtusus* (small and large) were strongly influenced by mean relative humidity and number of days without rainfall two months before the sampling time, two variables related to moisture. Soil moisture has been mentioned by Morrison et al. (2000) as one of the single best predictors of phorid abundance at one site in the United States. In general, high humidity would favor pupation and pupal survival of *Pseudacteon* flies (Ramirez 2004). These three species, plus *P. cultellatus*, also were favored by higher temperatures (historical minimum mean monthly temperature and mean temperature 2 mo before the sampling time). Annual activity of the rest of the species (all of them of large size) was not explained by the climatic variables analyzed.

**Daily Activity Pattern.** Seven species of *Pseudacteon* flies occurred at trays containing *S. invicta* workers exposed throughout the day at NADC and CBS. Species exhibited different activity patterns depending on the site or the time of day (Fig. 3). However, four species had similar behavior patterns in both sites (see correlation values in Fig. 3). Despite the similarity of annual activity patterns, daily patterns of *P. nudicornis* and *P. cultellatus* differed at each site. Fly abundance was higher close to dusk, whereas species diversity was highest at midday. Nearly three times as many flies were observed around sunset than at midday. This peak of vespertine abundance is largely due to the presence of *P. nocens* and *P. litoralis* (Fig. 3). Both species also were the only ones present at sunrise.

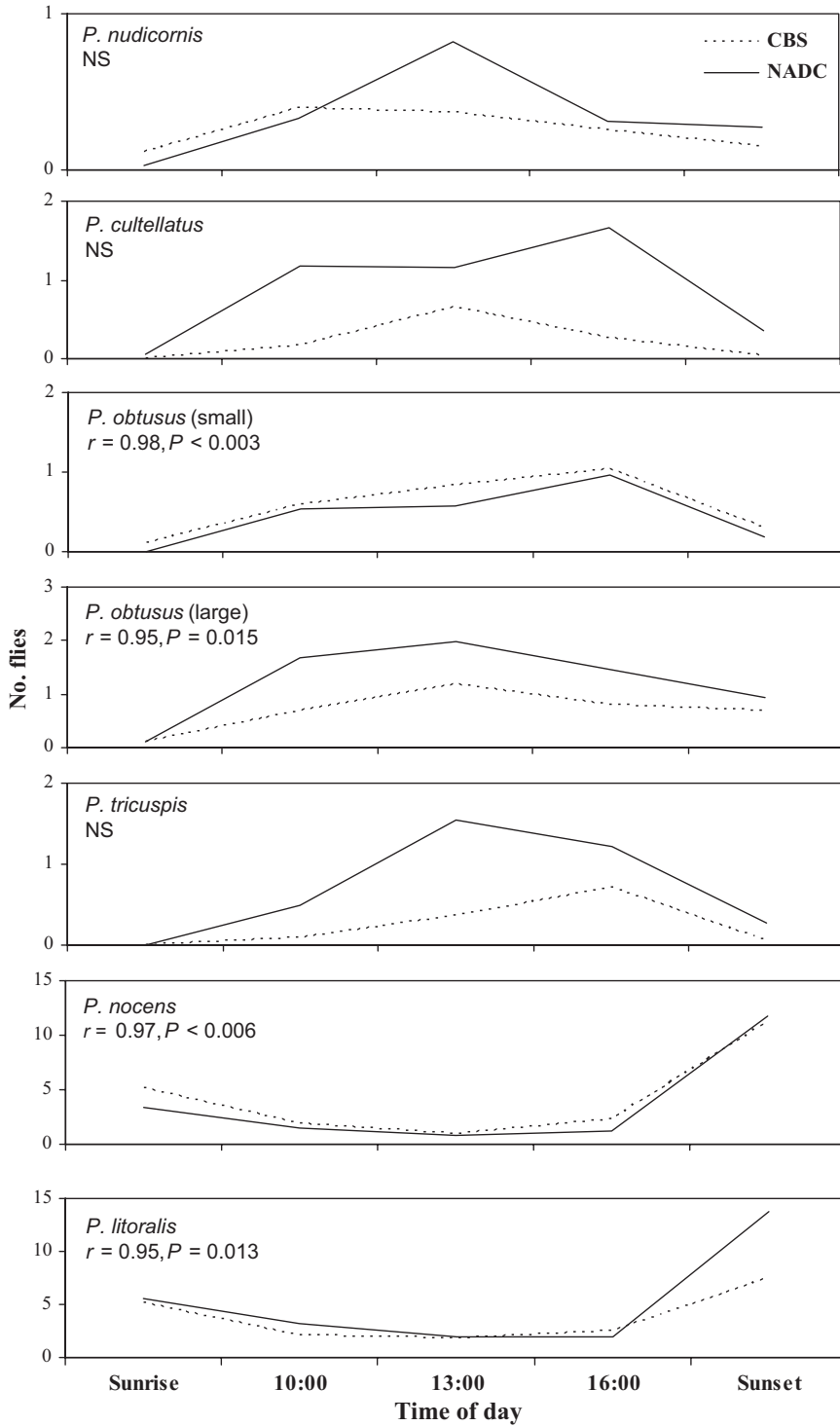


Fig. 3. Diurnal activity pattern of the seven *Pseudacteon* species studied. Note differences in scale of y-axes (mean number of flies per sampling effort). Statistical legends refer to the correlations of species activity patterns between sites.

Cluster analysis separated similar two groups of species in each site (Fig. 4). The first group corresponded to the “crepuscular species” (*P. litoralis* and *P. nocens*),

whereas the second group was compounded by another five “midday species”. In NADC, daily patterns of *P. litoralis* and *P. nocens* were very similar ( $r = 0.99$ ,

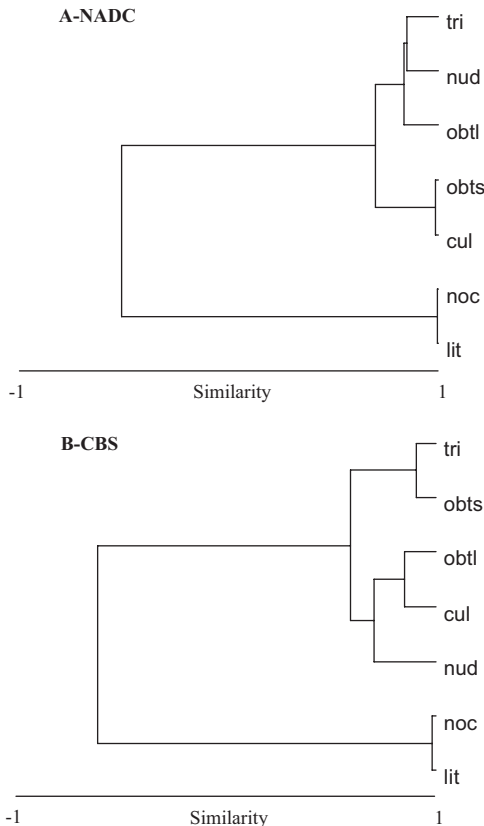


Fig. 4. (A and B) Relationship among the seven studied species in National Aquaculture Development Center (A-NADC) and Corrientes Biological Station (B-CBS) based on correlations of their daily activity patterns.

$P < 0.002$ ). Also, *P. cultellatus* pattern was very similar to the small *P. obtusus* ( $r = 0.99$ ,  $P < 0.002$ ). In CBS, daily fluctuation of *P. litoralis* and *P. nocens* also were very similar ( $r = 0.99$ ,  $P = 0.002$ ), whereas variation of *P. tricuspsis* was similar to the one of the small form of *P. obtusus* ( $r = 0.90$ ,  $P < 0.04$ ). The similarity of the circannual and circadian rhythms of *P. litoralis* and *P. nocens* matches with their morphologic similarity (Porter and Pesquero 2001).

Azzimonti et al. (2004) reported that *P. nocens* also was very active around midday in winter and fall in Santiago del Estero, which contrasts with the pattern observed in Corrientes. A possible explanation for this difference could be a lower mean temperature during the winter in Santiago del Estero ( $12.6^{\circ}\text{C}$  for July) compared with Corrientes ( $15.4^{\circ}\text{C}$ ) (De Fina 1992). *P. litoralis* was the most tolerant to low temperatures ( $16^{\circ}\text{C}$ ) and the small *P. obtusus* was the most tolerant to high temperatures ( $36.5^{\circ}\text{C}$ ). North American *Pseudacteon* flies have been reported as active at temperatures  $>37^{\circ}\text{C}$  (Morrison et al. 1999).

Daily abundance of two fly species was explained by some climatic variables measured at the time of the census. The abundance of large *P. obtusus* was strongly explained by the mean temperature at CBS ( $R^2 = 0.88$ ,

$P < 0.02$ ). At NADC, abundance of large *P. obtusus* was better explained by relative humidity ( $R^2 = 0.79$ ,  $P = 0.044$ ), whereas *P. tricuspsis* abundance (though marginally significant) by mean temperature ( $R^2 = 0.77$ ,  $P < 0.051$ ). *P. obtusus* (large morph) and *P. tricuspsis* were mentioned by Azzimonti et al. (2004) as the most active species during the hotter hours of the day in spring and summer in Santiago del Estero. Daily activity patterns of *P. tricuspsis* and *P. litoralis* described here (Fig. 3) were similar to that reported by Pesquero et al. (1996) for these same species, but attacking *Solenopsis saevissima* (Smith), in Brazil. They also found the mean peak of activity occurring around midday (7–9 h after sunrise) for *P. tricuspsis* and vespertine (2–4 and 10–12 h after sunrise) for *P. litoralis*. Moreover, Wuellner and Saunders (2003) studying North American *Pseudacteon* flies found that when the temperature is warmer, the flies occurred earlier in the morning and later in the evening.

All fly species studied in this work were considered by Folgarait et al. (2005) as species that occupy a wide range of habitats and climates (widespread species-group). This characteristic could suggest a low effect of the climate on their phenology. However, as in other studies (Pesquero et al. 1996; Morrison et al. 1999, 2000; Folgarait et al. 2003; Morrison and Porter 2005), climatic variables, such as mean temperature, relative humidity, and rainfall, and likely habitat type were able to explain, at least partially, phenological patterns of some species of the studied assemblages. Host activity patterns also could help to explain the fly activity patterns. However, host availability (foraging ants) did not explain native phorid fly abundance in the United States (Morrison et al. 2000). The wide range of daily variability in abundance observed in Corrientes also could be explained, as suggested by Morrison and Porter (2005), by the short life span of the adult flies (from 4 d at  $33^{\circ}\text{C}$  to 15 d at  $20^{\circ}\text{C}$  for *P. tricuspsis*; Chen et al. 2005), and differential emergence time of each fly species.

**Genetic Relationships.** The phylogenetic reconstruction revealed that two of the species sampled at CBS (*P. cultellatus* and *P. nudicornis*) seemed to be genetically quite similar to each other, but distant from all the other *Pseudacteon* sampled (Fig. 5). Although genetically related, these species did not seem to have a similar activity pattern, perhaps due to their low abundances. The exact relationship of these species to other *Pseudacteon* may be resolved by additional studies that include more extensive taxon sampling and greater amounts of DNA sequence data.

In this analysis, we also found less support for the monophyly of *P. obtusus* than reported in previous studies (Calcaterra et al. 2007), but this is almost certainly due to the much shorter length of DNA sequence used here. In this study, we were constrained to the 730 bp of COI sequence that was obtainable for some of the samples, whereas Calcaterra et al. (2007) were able to recover 1400 bp of COI and cytochrome *b* from all *P. obtusus* samples. However, the two *P. obtusus* groups that were recovered as well-supported monophyletic clades here were



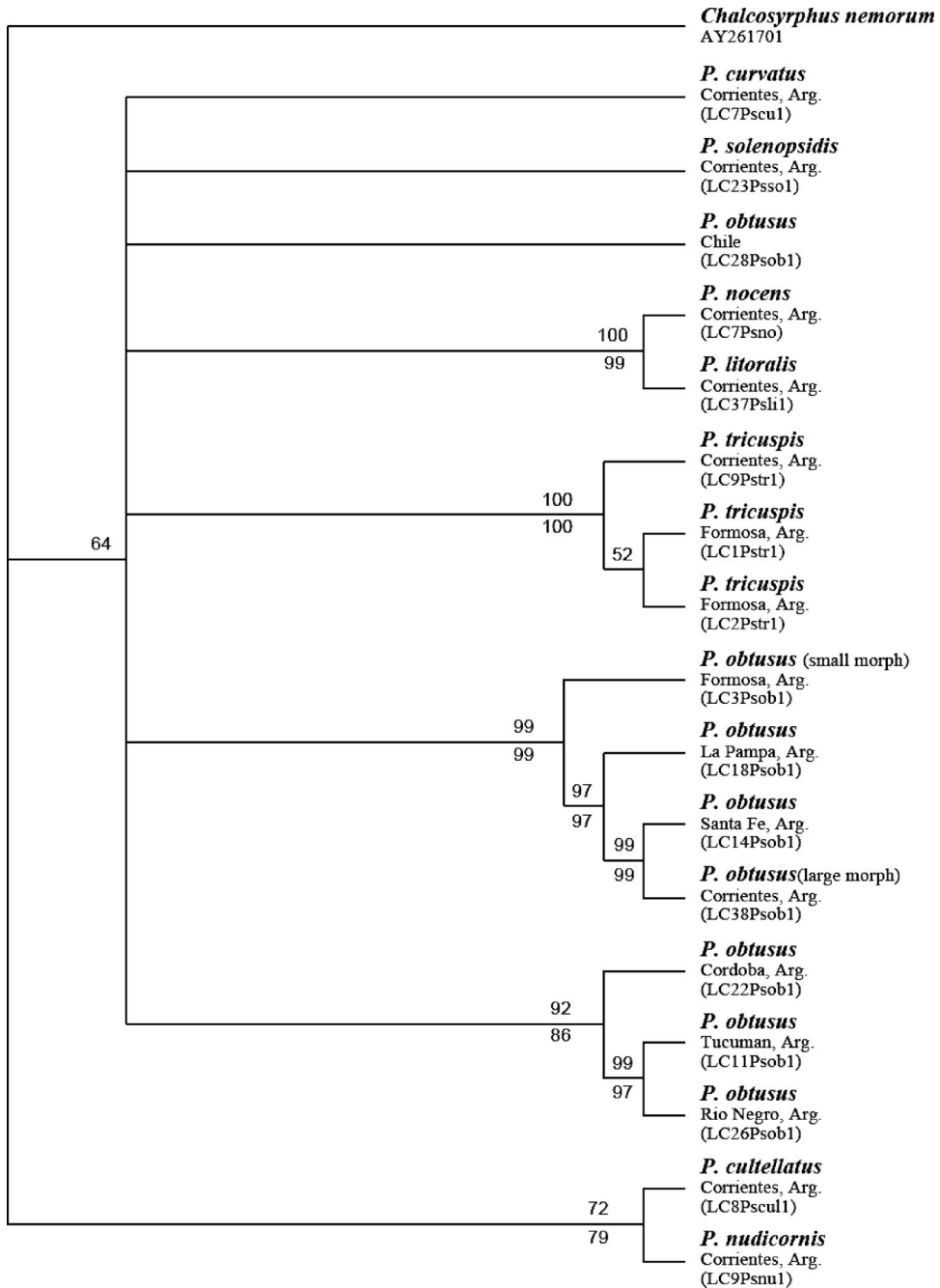


Fig. 5. Phylogenetic reconstruction of relationships among the assemblage *Pseudacteon* species at CBS. The tree is based on 730 bp of COI sequence. Each sample is labeled with the species name (bold), the collection location, and the individual sample's identification number (parentheses). A sequence from a syrphid fly, *C. nemorum*, was used as the outgroup. The maximum parsimony bootstrap values are shown above each branch; the maximum likelihood values are shown below each branch.

matched findings from the previous study (Calcaterra et al. 2007). The factor (climate, environment, geography, or host fire ant species) that explains the differentiation of these two groups is currently unknown (Calcaterra et al. 2007).

The *P. tricuspis* individuals collected in Corrientes formed a well-supported monophyletic group with two other *P. tricuspis* from a different site (Formosa, Argentina), located 105 km away (Fig. 5). Two other species at CBS, *P. nocens* and *P. litoralis*, were paired

together with high levels of bootstrap support, but the relationship of this clade to others was unresolved. Similarly, the relationship of *P. curvatus* and *P. solenopsidis* to the other *Pseudacteon* remained unresolved in this analysis. Although it seems clear that these species are more closely related to each other (and to *P. obtusus* and *P. tricuspsis*) than they are to *P. cultellatus* and *P. nudicornis* (Fig. 5), more precise estimations of their phylogeny will require a more thorough, taxonomically broad analysis.

For the most part, it seems that there is not relationship between phylogenetic proximity and activity patterns. For example, *P. cultellatus* and *P. nudicornis* were most genetically dissimilar from the other *Pseudacteon*, but they displayed daily activity patterns that were similar to that of *P. obtusus* (both large and small morphs) and *P. tricuspsis* (Fig. 4). In contrast, however, *P. nocens* and *P. litoralis* were both genetically and behaviorally similar, suggesting that their particular behavioral patterns may be a shared derived trait from a recent common ancestor.

**Natural Parasitism Rates.** Overall parasitism rates across all of the workers collected in the field were very low (0.24%). Only 739 pupae were found from 311,645 worker ants (Table 1). This is the first report of *S. invicta* colonies naturally attacked by *Pseudacteon* flies in its native Argentina. This rate was six-fold higher than that reported by Morrison and Porter (2005) for introduced populations of *S. invicta* parasitized by *P. tricuspsis*, 3–4 yr after its release in the United States ( $\approx 0.04\%$ ).

Parasitism rates were very variable among sites and months (Table 1). High variability also was observed among colonies. Due to this variability, a pattern describing the dynamic of parasitism could not be found. The highest parasitism rate pooling the four places was in spring (0.51%) or per site pooling the three seasons was for MC (the habitat with the most complex vegetation structure) with 0.50% (Table 1). The relatively high parasitism rate recorded in November 2004 (spring) in CBS, for example, matched with the highest fly abundances recorded in that same site in November 2005, supporting the idea that the spring is the more favorable season for phorid flies in Corrientes. The maximum parasitism rate observed (or mortality caused by flies) in this season (0.5%) does not seem to be high enough to cause an important (and direct) effect on *S. invicta* populations.

The highest seasonal parasitism rate per site and colony also was for MC (1.16 and 2.81%, respectively) during the spring. This agrees with the findings by Morrison et al. (1997) in the United States, where up to 2.42% of the colonies of *S. geminata* were naturally parasitized by two North American *Pseudacteon* species during the fall. Parasitism rates of the phorid *Apocephalus* attacking *Pheidole dentata* Mayr's workers were <5% in Texas and <2% attacking *Pheidole titanis* Wheeler in Mexico (Feener 1988). A much lower field rate by colony (0.058%) was reported by Morrison and Porter (2005) for *S. invicta* parasitized only by *P. tricuspsis* in the United States. However, parasitism rates in this case were only determined from workers col-

lected in the month with highest abundance of flies (November), whereas in our case it was obtained from three different seasons (spring, summer, and fall).

Pupae were found in dead workers from day 2 after the colonies were collected in the field. If parasitism of fire ant workers is constant throughout the time in the field, we would have found pupae from day 1 after collection. Our finding contrasts with the reported by Morrison and Porter (2005) for *P. tricuspsis* in the United States, because they found pupae at day 9 after collection. This difference could be mainly due to the different number of species recorded in each study, with their different developmental times. Nine species were recorded in our study, whereas only one species was recorded in the United States. Other fly species absent in the United States could have pupated within the first 8 d, as observed in our study.

Of the 739 pupae found, only 223 (30%) adults of nine species emerged, the eight mentioned above, plus *P. solenopsidis* (Table 2). This ninth species emerged from the colonies collected in the habitat with a more complex vegetation structure (MC), which could reveal some kind of preference for habitat. From MC emerged the 61% of the flies belonging to eight species (*P. tricuspsis* was absent), followed by CU with the 20% of the flies from seven species (Table 2). The percentage of emerged adults from the total number of recorded larvae and pupae (success rate) were low: 26.1, 31.8, and 41% for November, February, and April, respectively.

A few more males (116) than females (107) emerged resulting in an overall field sex ratio (secondary sex ratio) of  $\approx 1:1$ . Surprisingly, the highest number of emerged flies (considering only the females) corresponded to *P. nudicornis* (35/107), followed by *P. litoralis* and the small form of *P. obtusus* both with 18 females (Table 2). Nightly, 3% of the emerged *P. nudicornis* adults were from MC. *P. nudicornis* was the least abundant species in previous field collections from fire ant mounds (Calcaterra et al. 2005) and in the trays containing fire ant workers exposed throughout the year (Fig. 1). The reasons for this difference in abundance are unknown. However, a differential response to the rearing condition (mainly humidity) could have favored the survival of *P. nudicornis*. Humidity loss at pupation (this process requires a relative humidity of  $\approx 100\%$ ) also could have caused the low success rates recorded in this work.

This is the first study that reports detailed patterns of activity of the red imported fire ant decapitating flies in their native land, relationships among fly species based on their activity patterns and genetic sequences, and four climatic variables explaining annual and/or daily variations in five of the seven fly species of the assemblages. The numerical dominance of *P. nocens* and *P. litoralis* in the studies assemblages could be explained by their apparent escape from environment constraints because their annual and daily variations were not associated with climate. Additionally, this work reports for the first time natural parasitism rates of *S. invicta* from multiple sites and seasons in its homeland. Although fluctuations in seasonal patterns

**Table 2.** Number of adult flies emerged in Machuca Campground (MC), Concepción del Uruguay (CU), Corrientes Biological Station (CBS), and Herradura (HF)

Date and site	No. <i>Pseudacteon</i> females <sup>a</sup> (males)									Total
	<i>nud</i>	<i>lit</i>	<i>obts</i>	<i>obtl</i>	<i>noc</i>	<i>cul</i>	<i>cur</i>	<i>sol</i>	<i>tri</i>	
Nov. 2004										
MC	20	3	1	1	1	6	1	1		34 (57)
CU	1		1				4		2	8 (9)
CBS		6	6	2						14 (11)
HF	1	3	-							4 (7)
Subtotal	22	12	8	3	1	6	5	1	2	60 (84)
Feb. 2005										
MC				4	1	1	1			7 (3)
CU		1		1	2					4 (4)
CBS			5	1						6 (4)
HF										0 (0)
Subtotal		1	5	6	3	1	1			17 (11)
April 2005										
MC	12	2	2		3	1	1	1		22 (13)
CU										0 (0)
CBS	1	3	2		1					7 (6)
HF			1							1 (2)
Subtotal	13	5	5		4	1	1	1		30 (21)
Total	35	18	18	9	8	8	7	2	2	107 (116)

<sup>a</sup> *Pseudacteon* species: *nud*, *nudicornis*; *lit*, *litoralis*; *obts*, small *obtus*; *obtl*, large *obtus*, which matching original species description (Borgmeier 1963); *noc*, *nocens*; *cul*, *cultellatus*; *cur*, *curvatus*; *sol*, *solenopsidis*; *tri*, *tricuspis*.

of parasitoid fly abundance are complex to explain because they depend on, among other factors, intrinsic fly physiology (genetically based), the environment, their host species and habitat, the information obtained in this study will be useful to select new fly species (and biotypes) for potential field release, to predict their behavior, and to estimate the magnitude of their detrimental effect as classical biological control agent against the red imported fire ant in the United States.

### Acknowledgments

We thank Juan Briano, Sanford Porter, and Lloyd Morrison for the valuable comments and suggestions to improve the manuscript. We thank especially Juan Pablo Livore, Laura Varone, Sonia Cabrera, and Joaquín Sacco for help during field and laboratory work and/or data processing. We also thank the logistic support provided by staff at the Corrientes Biological Station and National Aquaculture Development Center. This work was supported, in part, by grants to N.D.T. from the United States Department of Agriculture (NRI-CGP-2004-35302-14865) and the California Department of Consumer Affairs-Structural Pest Control Board.

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Received 30 August 2007; accepted 14 December 2007.